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Abstract

Phosphorus (P) is an important element for crop production. A low concentration limits crop growth whereas the residual quantity after fertilization contributes to eutrophication of surface waters. A key to success in the soil P management is its accurate estimation and potential supply to the plant. The objective of this project was to compare the soil P extractability between the Pi- filter strip method and P depletion without a sink (0.01 M $CaCl_2$). The results were compared to those obtained using ammonium lactate (AL), Olsen (OL) soil P tests. The results showed that the efficiency of different methods decreased in the order Pi (62-85%) > OL (25-40%) > 0.01 M $CaCl_2$ (8-24%) of the total P extracted by AL. A strong correlation (r = 0.99) was obtained between the pairs (Pi; AL), (Pi; OL), and (OL; AL). Further, it was found that all methods were equally well correlated (the r was between 0.88 and 0.90) with the depletion method. The soil with the highest P adsorption capacity had higher values of P extracted by the AL, Pi and OL methods but was lower in 0.01 M $CaCl_2$ extractable P. The results indicated that in the soils studied, the efficiency of the Pi-filter strip method in comparison to the routine P tests (AL and OL) was not attributed to the soil properties. It was rather attributed to the length of soil P desorption time and the number of filter strips.

Popular science summary

Phosphorus (P) is one of the major nutrients for crop production. Therefore, P is important for ensuring food security but it is a non-renewable resource like oil. An insufficient amount of P in soil may limit the plant growth. Historically, organic material such as animal manure, industrial organic wastes, guano (bird excreta deposited over the past millennia) was used to improve the soil fertility. Since the mid-19th century the use of P fertilizers obtained from mining phosphates reserves has become more preferred. The reserves may be depleted in 50 to 100 years. Another serious P-related problem is that over-fertilization may promote P leaching thus contributing to surface water eutrophication.

P can exist in three pools in soil, referred to as solution, active and fixed P. The soil solution is the aqueous liquid phase and its solutes. P in solution represents a readily available P but it is a very small pool. During plant growth, P in solution is continuously removed by the plants and replaced by P from the active pool (attached to the soil particles). The growing vegetation would quickly deplete the solution Pool if the soil P becomes unavailable (fixed-P). The P fixing compounds include aluminum, iron and calcium metals. Therefore, the soil P supply potential to the solution is what limits the plant access to P.

The best way to maintain a high crop yield and stop surface water pollution by residual P loss (eutrophication) is through determination of the adequate amount of plant available P prior to fertilization. More than fifteen methods to measure available P are now available but they all result in large variation between each other or internally. In one recent method, filter strips are coated with iron (hydr)oxides and used to remove P from the soil solution. This method can be used to assess the soil P release characteristics over an extended time thus imitating the crop uptake of P during the growing stages.

A consecutive removal of P using strips was investigated on two soils included in the Swedish long-term fertility experiments. This method was compared with three commonly used methods; the ammonium lactate- (AL, the Swedish standard method), Olsen- and Calcium chloride-methods. The results suggested that more P is fixed in soils after the long-term fertilization. The first three methods showed that soils with more aluminum and iron content were able to sorb more active P which can be releasable to the solution. These three methods seemed also to have similar potential to measure the quantity of P that can replenish the solution P. In contrast, calcium chloride method showed that soils with less aluminum and iron content can have more P in solution. This is very low and can be considered as only available in early stages of growing crops. It was also concluded that the number of strips and the length of time in contact with the soil suspension can explain the difference between Pi-filter strip, AL and OL methods. A more clear assessment of the efficiency between these methods might need the inclusion of soils covering a wide range properties ranging from acidic to alkaline soils.

Populärvetenskaplig sammanfattning

Fosfor (P) är ett makronäringsämne nödvändigt för växtproduktion. På grund av detta är det en viktig resurs för att säkra världens matförsörjning, men precis som oljan är den ändlig. En alltför låg koncentration av P i jorden kan leda till begränsningar av växternas tillväxt. Historiskt har organiskt material som t.ex. stallgödsel, industriavfall och guano (avföring från fåglar som anrikats över århundraden) använts för att förbättra jordens bördighet. Från 1900-talets mitt har dock användandet av mineralfosfor utvunnet från gruvdrift ökat alltmer. De reserver av fosforrika mineral som finns kan komma att utarmas om cirka 50-100 år. Ett annat allvarligt problem relaterat till P är att överdriven gödsling kan leda till P-läckage och därmed bidra till övergödning av ytvatten.

P i jord kan existera i olika “pooler” vilka kan delas in i P i marklösningen, aktivt P och fixerat P. Löst P representerar den P-fraktion som är direkt tillgänglig för växter, och är en väldigt liten pool. Under växtsäsongen blir löst P kontinuerligt upptaget av växter, och den lösta poolen blir då påfylld av P från den aktiva poolen. Den växande vegetationen kommer snabbt att tömma den lösta P-poolen om allt P i jorden blir oåtkomligt (fixerat P). Fixerat fosfor kan finnas som fosfor starkt bundet till aluminium- och järnhydroxider samt som kalciumfosfater (apatit). Jordens potential att leverera P till växter är begränsad framför allt av dess potential att leverera P från den aktiva poolen till den lösta.

Den bästa strategin för att bibehålla höga skördar samt förhindra P läckage är att mäta mängden vättillgängligt P innan gödsling. I nuläget finns det mer än femton olika metoder för detta, men de skiljer sig mycket från varandra. I en lovande ny metod används filterpapper behandlade med järn(hydr)oxid för att ta upp P från en jordsuspension. Denna metod kan användas för att undersöka jordens frigörelse av P som funktion av tiden genom att imitera upptaget från rötterna hos en växt under tillväxt.

Jordens förmåga att leverera P studerades genom användandet av impregnerat filterpapper studerades för två jordar från de svenska bördighetsförsöken. Denna metod jämfördes sedan med tre ofta använda metoder, sur ammoniumacetatlaktat (AL, svensk standardmetod), Olsen (OL) och kalciumklorid. Resultaten antyder att mer P finns bundet i jordar som gödslats under en lång tidsperiod. De tre först nämnda metoderna visade också på att jordar med högt innehåll av järn och aluminium kan binda mer aktivt P som sedan kan frigöras till marklösningen. Dessa tre metoder tycks också ha liknande potential för att mäta den mängd P som kan frigöras till marklösningen. I motsats till detta visade kalciumklorid-metoden att jordar med lägre innehåll av järn och aluminium kan ha mer löst P. En slutsats är också att antalet filterpapper samt kontakttiden med jordsuspensionen kan förklara skillnaden i resultat mellan filterpappermetoden, AL och OL. En tydligare utvärdering av skillnaden i effektivitet mellan dessa metoder kommer att behöva inkludera ett bredare urval av jordar med olika egenskaper, från sura till alkaliska.

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1. Introduction

Phosphorus (P) is one of the essential elements for life. A sufficient amount of P in soil should be accessible to photosynthetic organisms to sustain a secondary production. However, the plant growth may be limited by the low concentration and solubility of soil P, partly because of depletion in soil without replenishment (Sanchez, 2002), or high P fixation as a result of strong weathering (Fink *et al.*, 2016). Historically, agricultural ecosystems were adapted to locally produced P from organic matter (Smit *et al.*, 2009). Since the mid-19th century, the use P fertilizer from locally produced organic matter became less preferred. The world had then become addicted to artificial P fertilizers as a result of rapid population growth. Consequently, the current reserves may be depleted in 50-100 years (Cordell *et al.*, 2009). Agricultural soils, especially in developed countries, have been extensively fertilized, leading to a build-up of P in soil (Breeuwsma & Silver, 1992; Koopmans *et al.*, 2001). As a result, the loss of residual P from agricultural soils is among the most serious threats to surface water environment (Breeuwsma & Silver, 1992; Daniel *et al.*, 1998, Daniel *et al.*, 1998; Djodjic & Bergström, 2005). The solution P is continuously removed by the crops or lost through the surface runoff or subsurface drainage. This fraction is replenished by the sparingly soluble pool which is adsorbed on soil particles or precipitated with soil metals (Sparks *et al.*, 1996). Therefore, a deep understanding of the mechanisms of P release through the soil-solution-plant system is required to better understand its availability in soil.

Late the 19th century, P availability in soil has been extensively studied and now, more than 15 soil P tests are available (Neyroud & Lischer; 2003Wuenscher *et al.*, 2015; van Rotterdam *et al.*, 2012). The most recent test is based on P removal from the soil using iron (hydr)oxide impregnated filter paper strips (Pi-filter strip method) (Chardon *et al.*, 1996). It is an advantageous method given that it meets criteria of being most preferred for extracting potentially available P. (Van Rotterdam *et al.*, 2012; van Rotterdam *et al.*, 2009; Chardon *et al.*, 1996). Moreover, the solution P can be simply measured using 0.01 M $CaCl_2$ method, which gives low P values (Houba *et al.*, 2000). Both the soil solution P and potentially extractable P are very important in predicting the P supply potential (van Rotterdam *et al.*, 2012; Hinsinger, 2001).

The most commonly used tests include the ammonium lactate (AL), Olsen (OL), and $CaCl_2$ methods. AL has been adopted as a standard method in many European countries such as Sweden, and the OL method is used in a number of other European countries (Otabbong *et al.*, 2009).

However, one criticism of much of the literature on soil P testing is that all tests give results with large variation of plant available P. Therefore, there is a lack of consensus in predicting the long-term P supply based on single test methods (Neyroud & Lischer, 2003; van Rotterdam *et al.*, 2012; Wuenscher *et al.*, 2015).

In this work, a set of experiments was designed to study the consecutive desorption of P from soil using Pi-filter strips as P sinks (Pi filter strip method) (van Rotterdam *et al.*, 2012; van Rotterdam *et al.*, 2009; Chardon *et al.*, 1996) and compared to a desorption method without the use of a sink. The latter method will be referred to as the soil P depletion using 0.01 M $CaCl_2$. The data from the AL- (Egner *et al*, 1960), OL- (Olsen *et al*., 1954) and the standard 0.01 M $CaCl_2$- methods (STD $CaCl_2$) (Houba *et al.*, 2000) were used to provide an integrated interpretation of P results. The latter is a standardized method to determine P in soil solution where a ratio of soil to solution of 1: 10 and the shaking time of 2 h are considered.

Results from the standard method for characterizing the soil solution P will be referred to as initial P concentration. $CaCl_2$-P will be solely used for from a depletion experiment P results. The notation Pi-P will be used for P adsorbed to the Pi- filter paper strips whereas AL-P and OL-P will be used for P extracted by the AL and Olsen methods, respectively.

1.1 Objectives and hypotheses

The overall objective of this project is to compare the soil P extractability between the Pi- filter strip method and the P depletion without a sink (0.01 M $CaCl_2$ depletion method), to better understand the development of P desorption from the soil. The stated objective will be accomplished by fulfilling the following research specific objectives:

1. Assess the sequential extraction of soil P using Pi-filter strips in comparison with the soil solution P as determined by 0.01 M $CaCl_2$ depletion method.
2. Test the Pi-filter strip method with two routinely used soil P tests; AL- and OL-methods.
3. Evaluate the effect of various soil fertility levels on P availability in soils using Pi-, AL-, OL- and 0.01M $CaCl_2$- methods.

Hypotheses tested were:

1) The amount of P adsorbed to individual Pi-filter strips will be decreasing over time as a result of successive depletion of the soil-adsorbed P from the soil.
2) The P concentration in solution will be decreasing as a result of both removal and a slowdown of P desorption rate.
3) Soils with high concentration of Al/Fe (hydr)oxides will release more soil-adsorbed P during the Pi-filter strip experiment.

2. Literature review

This chapter comprises the information on the main P species in soil, the theory about P sorption processes using the Pi-filter strip method in the soil-solution-strip system and an overview of routine soil P tests.

2.1 Phosphorus forms in soil

2.1.1 Dissolved P

P in soil, soil solution, surface runoff and subsurface flow is present in both inorganic and organic forms. Figure 1 illustrates the pools and processes that control P species. P is recycled within various soil components that will be referred to as soil P pools in this report. Inorganic P ranges from 35 to 70 % of the total P in most soils (Shen *et al.*, 2011). In terms of concentration, estimated P in soil solution (P2) ranges from 10^{-8} M (0.31 µg L^{-1}) in extremely deficient tropical soils to 10^{-6}M (31 µg L^{-1}) in P deficient soils and up to 10^{-4} M (3100 µg L^{-1}) in P-rich soils (Syers *et al*., 2008). The most abundant P species in solution is $H_2PO_4^-$ at pH 6, then HPO_4^{2-} becomes more abundant as the pH increases, whereas and PO_4^{3-} may be present in solution at extremely high pH (Kruse *et al.*, 2015).

2.1.2 Precipitated P

Phosphorus becomes geochemically active through the weathering of primary minerals such as apatites (P1). Since the weathering process is too slow to maintain soil fertility in an agricultural system where crops are continuously harvested (thus removing P from the system), P fertilizers are applied. This results in increase of the available P in solution which can be leached, removed by crops and microorganisms, or lost through surface water runoff or transformed into secondary minerals (Smeck, 1985; Shen *et al.*, 2011). Phosphate ions in the soil solution precipitate with metals such as Ca, Al, and Fe to form a wide range of secondary minerals. The precipitation and dissolution of these minerals depend on a number of factors including pH, the concentration of phosphate ions and of those responsible metals. In acidic soils, phosphate ions precipitate with Al/Fe and their dissolution increases as the pH goes up whereas the solubility of Ca-phosphates decreases above pH 8 (Hinsinger, 2001; Shen *et al.*, 2011).

2.1.3 Soil-adsorbed P

Adsorption-desorption are the major processes controlling the solution P concentration in most of the soils. P in soil is adsorbed on clay particles, Al and Fe (hydr)oxides (Hinsinger, 2001; Eriksson *et al.*, 2015). These two reactions depend on a number of factors such as pH and the variable charge. The zero point of charge of Al and Fe (hydr)oxides ranges generally between pH 7 and 10. Therefore, they have a positive variable charge in most of the soils. As the pH goes down, the positive surface charge increases, thus enhancing PO_4^{3-} adsorption to the soil. Reversibly, P desorption is induced by low P concentration in solution or increase of the concentration of competing anions (Hinsinger, 2001). Organic P is also important for the solubility of through mineralization-immobilization. (Eriksson *et al.*, 2015).

In summary, in acid and neutral soils, the solubility of P is maily controlled by the adsorption/desorption processes whereas the precipitaion-dissolution processes become more important in alkaline and calacareous soils.

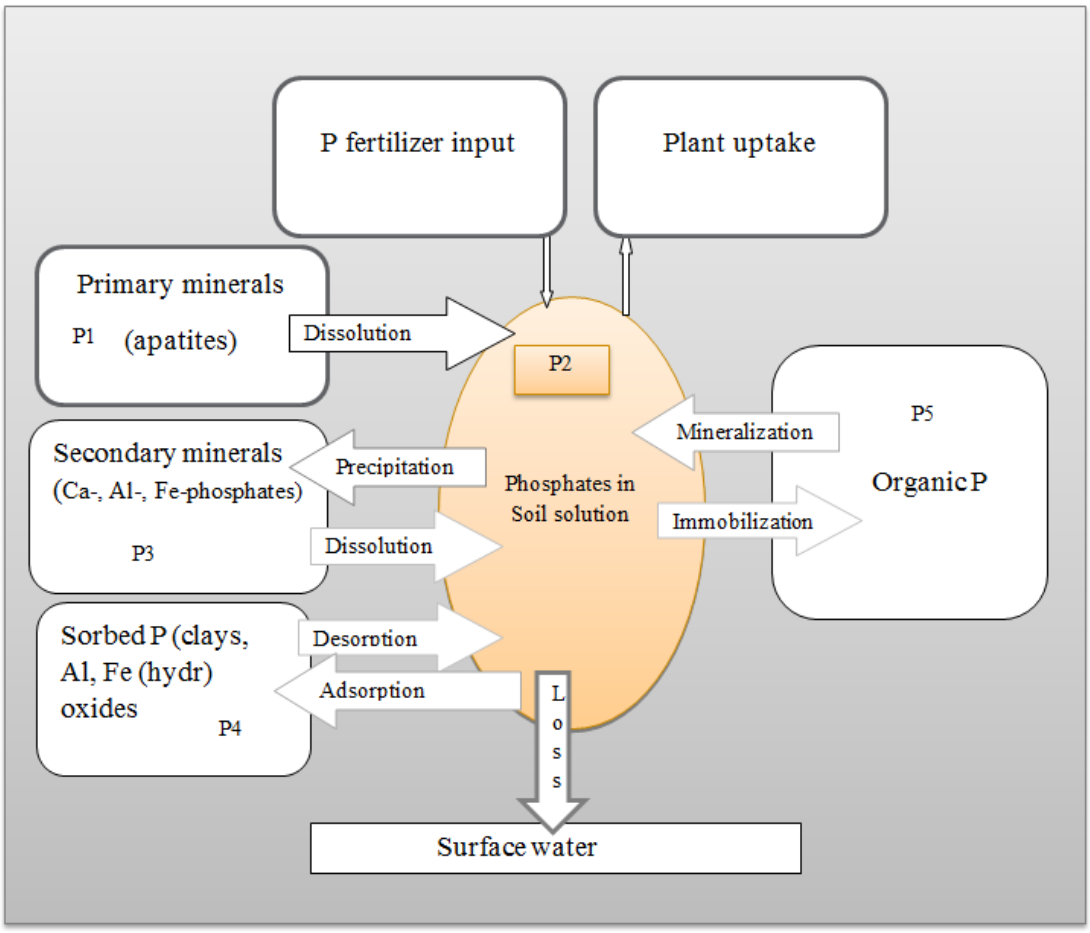


Figure 1: Phosphorus transformation in soil ecosystems.

2.2 Determination of P available in soil

2.2.1 Soil P determination using the Pi filter strip method

The Pi filter strip method has been shown to overcome a number of challenges in determination of P availability in soil. These challenges include accurate measurement and applicability to all soil types. The method is based on adsorptive extraction of P from the soil using filter paper strips coated with iron (hydr) oxide. Special filter papers are prepared and cut into strips which are then shaken with a soil suspension hence removing P mobilized into the solution from soil. Recently, researchers have shown that it is the best method which is useful to study P release characteristics over time, therefore mimicking plant roots uptake. (van Rotterdam *et al.*, 2012; van Rotterdam *et al.*, 2009; Chardon *et al.*, 1996). The principles of the Pi-filter strip method are briefly summarized in the following section.

The Pi strips consist of reactive sites which are similar to those of Fe (hydr) oxides in the soil. Under the prevailing conditions, P is transferred throughout the system as illustrated in Figure 2. A Pi strip is immersed into the soil suspension. Both desorption (1) and adsorption (3) rates may limit the amount of P removed from the solution. In the presence of a sink such as a Pi strip, P in the soil solution is transferred through adsorption (3) to the Pi strip, decreasing the concentration in solution. In this situation, P concentration may become negligible thus initiating P desorption (1). Therefore, in such a situation, the P desorption kinetics is limiting. On the other hand, equilibrium between solid and solution P is constantly attained whereas the equilibrium between the solution and the strips had not yet reached. Therefore, P transfer to the Pi strips is controlled by the adsorption kinetics of the Pi-filter strips. In this situation, the P concentration in solution is a function of the P desorption isotherm of the soil (van .Rotterdam *et al.*, 2009). The number of strips used could determine which rate is limiting the transfer of P. A number of reports revealed that the maximum desorption is shortly attained when 4 to 5 strips are used consecutively (Van. Rotterdam *et al.*, 2009; Chardon *et al.*, 1996; Lin *et al*., 1991).

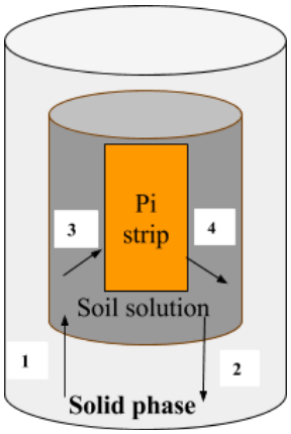


Figure 2:A diagram representing a soil-solution-Pi-strip system including adsorption and desorption rates.

2.2.2 Standard soil P tests

Numerous methods are available for soil P testing. Details on the two most commonly and widely used tests, AL and Olsen, are summarized in Table 1.

For a soil P test to adequately measure and predict the plant available P, it should respond to the soil characteristics in similar way as plant uptake (Sparks *et al*., 1996) . Chardon *et al*., (1996) and Sparks *et al*., (1996) categorized the tests into 5 groups. The 1st group includes shaking the soil with water or diluted salt solutions such as $CaCl_2$ to represent readily available P to plants; 2nd, diluted weak and strong acidic extractants (e.g ammonium lactate method); 3rd, shaking the soil with buffered alkaline solutions (e.g Olsen method); 4th soil P determination using Isotopic dilution and the 5th group includes the use of P sinks (anion exchange resin and iron (hydr) oxide filter paper strips). However, the complexity of P chemistry in soils hampers a consistent assessment of the size of bioavailable P, since the P determination is carried out using different procedures. The most commonly used tests are described below.

Table 1: Description of the AL and OL soil-P tests in various European countries.

Country	Method	Chemical extractant	Reference	Soil types
Belgium (Flanders) Hungary Lithuania Norway Slovenia Sweden	AL	1:20, 0.1 M ammonium lactate + 0.4 N acetic acid, pH 3.75, 2 h shaking	Egnér *et al*., (1960)	All soils
Denmark England France Italy Spain	OL	1:20, 0.5 M sodium bicarbonate, pH pH 8.5, 30 min shaking	Olsen *et al*., (1954)	Calcareous Extended to all soils

2.2.2.1 P extraction with AL and OL methods

AL has been primarily used as a standard in many European countries such as Belgium, Hungary, Lithuania, Norway, Portugal, Slovenia and Sweden (Table 1) (Jordan-Meille *et al.*, 2012).The reason why this method was adopted in Sweden is because the long-term soil fertility has been monitored based on this method. Moreover, it has been substantially used in the soil classification, teaching and other research projects. OL method has been mostly used to provide a helpful interpretation of existing standard (routine) methods in some European countries, such as Denmark, England, France and Italy. The AL extraction is based on shaking the soil with a mixture of ammonium lactate and acetic acid solution. The anion lactate is specifically bound to the Al and Fe (hydr)oxides in soil, thus enhancing a release of P adsorbed to these (hydr)oxides (Jordan-Meille *et al.*, 2012). Moreover, AL can release insoluble P bound to Ca-phosphates in alkaline and calcareous soils (Jordan-Meille *et al.*, 2012; Otabbong *et al.*, 2009). The OL test was developed by Olsen, (1954) to measure the P availability in calcareous soils. It consists of 0.5 M $NaHCO_3$. The solubility of soil phosphates in acid and neutral soils is thought to occur through competitive complexation of extracting anions with Al^{3+} and Fe^{3+} thus increasing the concentration of phosphates in solution. With the OL method, anions (HCO_3^{2-} and CO_3^{2-}) precipitate with $Ca^{2+,}$ releasing P in calcareous soils. Therefore, this method is applicable for both acid and calcareous soils whereby HCO_3^- ions replace adsorbed PO_4^{3-} whereas Ca^{2+}

precipitates with anions (bicarbonates) at high pH. Moreover, as the pH is usually increased up to 8.5 in the OL method, negative surface charge results, reducing the binding of negatively charged phosphate ions (Jordan-Meille *et al.*, 2012)

In short, the two chemical tests have been reported to be applicable to all soil types, but AL may overestimate the bioavailable-P in alkaline and calcareous soils as it can dissolve insoluble Ca phosphates (Olsen *et al*., 1954; Sparks *et al*., 1996; Jordan-Meille *et al.*, 2012).

2.2.2.2 Soil phosphorus extraction with 0.01 M $CaCl_2$ solution

The soil solution P is referred to as P intensity and represents a small P fraction which is easily available (Sánchez-Alcalá *et al.*, 2014). This pool is usually determined using $CaCl_2$ extraction (Sparks *et al.*, 1996). The extractant solution nearly maintains the original conditions of the soils. In other words, it often consists of more or less the same ionic strength as that in most soils. The optimum concentration that has been used the most is 0.01 M (Sánchez-Alcalá *et al.*, 2014; Houba *et al.*, 2000). The other factors considered in this P testing are the soil to solution ratio (SSR) as well as the shaking time. In a standardized experimental setup described by (Houba *et al.*, 2000), a ratio of 1 g of dry soil to 10 mL solution is used. This leads to a good performance in predicting the plant P intensity at the initial stage (Sánchez-Alcalá *et al.*, 2014).This statement did not rely on quantitative analysis since no analysis was done with a specific focus on the effect of varying the ratio.

Several researchers have compared different soil P tests. For the four soil P tests described above, it was found that the P values decreased in order: AL-P > OL-P > P-Pi >$CaCl_2$-P (Neyroud & Lischer, 2003; Wuenscher *et al.*, 2015) studies used 16 h for extracting P using Pi-strips. It was also reported by Indiati & Singh (2007) that P extracted by Pi-filter strips within 16 h was lower than OL-P. The same trend as well, was revealed by Otabbong *et al.*, (2009) reporting more P extracted by AL method than P extracted under OL-method procedures. Therefore, there could be noted that the time for extracting P using Pi-filter strip method may change this trend when it is lengthened (Chardon *et al.*, 1996).

In summary, the intensity of the soil is referred to as P extracted by a diluted $CaCl_2$ solution during the depletion experiment. The AL and OL methods can determine the potentially available P (quantity), hence extracting more P. However, the Pi-filter strip method can determine P that may become available over time.

3. Materials and Methods

3.1 Site location and land use

The soil samples were collected in 2015. The sites studied are Ekebo and Fjärdingslöv, two of the twelve sites included in the Swedish long-term soil fertility experiments. They are located in South Sweden, Skåne County. The experiments started in 1957 and the sites have been used for cultivation since late 19th century. Three distinct experimental treatments have been considered for monitoring the soil fertility. These include 1) crop rotation with and without the application of animal manure, 2) the application of phosphorus/potassium (P/K) fertilizers and 3) nitrogen (N) fertilizers at different application rates; 0, 1, 2 and 3. The samples analyzed were from the plots receiving 150 kg N ha^{-1} year^{-1}. These are "A3", samples without K and P fertilizers and "D3" where P/K fertilizers are applied to replenish the amounts removed by harvest plus extra 30 kg P ha^{-1} year^{-1} and 80 kg K ha^{-1} year^{-1} (Carlgren & Mattsson, (2001).

3.2 Soil description and P status

The collected soil samples were air dried, crushed and passed through a 2 mm sieve. Ekebo has loamy soil texture whereas Fjärdingslöv consists of sandy loam with clay accumulation in the subsurface profile. Being non-calcareous, the soil samples collected from both sites have pH around neutral. The organic carbon content is higher at Ekebo and both soils are free of calcite. The clay mineralogy in the top soil is dominated by expandable minerals (smectite) especially the soil from Fjärdingslöv followed by Mica. Despite low levels of kaolinite at both sites, it is relatively higher in soils of Ekebo (Table 2).

Table 2: Physico-chemical properties of the soils from Ekebo and Fjärdingslöv (Eriksson et al., 2015; Kirchmann et al., 1999). The pH and particle size data were provided by the supervisors.Al-ox and Fe-ox are oxalate extractable aluminum and iron.

		Particle size	PH (H_2O)	Org C	$CaCO_3$	Al-ox	Fe-ox	Total P
Sample ID	Texture	% < 2 mm		%		mmolkg^{-1}		
Ekebo								
EK A3-36	Loam	93.2	6.86	2.32	0.05	63.7	41.5	4.5
EK A3-55	Loam	84.3	7.09	2.32	0.05	63.7	41.5	4.5
EK D3-47	Loam	87.8	6.84	2.55	0.14	94.1	39.9	16.1
EK D3-62	Loam	94.0	7.27	2.55	0.14	94.1	39.9	16.1
Fjädringslöv								
FJ A3-36	Sandy loam	94.5	6.98	1.23	0.17	32.6	34.0	2.3
FJ A3-55	Sandy loam	92.4	6.63	1.23	0.17	32.6	34.0	2.3
FJ D3-47	Sandy loam	94.5	6.87	1.37	1.9	34.1	31.9	11.6
FJ D3-62	Sandy loam	94.3	6.71	1.37	1.9	34.1	31.9	11.6

3.3 Soil phosphorus extraction

In this work, a continuous P removal from the soil was studied by using Fe (hydr) oxide impregnated filter paper strips (van Rotterdam *et al.*, 2009). Furthermore, a soil solution depletion experiment was performed using the 0.01 M $CaCl_2$ test procedures (modified from Houba *et al.*, 2000). Moreover, the P analyses from the Pi-strips experiment was performed using the Thermo Fisher discrete analyzer (Gallery plus) available in the laboratory of the Department of Aquatic Sciences and Assessment at Swedish University of Agricultural Sciences (SLU). The $CaCl_2$ extract was analyzed with a Seal Analytical AA3 Autoanalyzer available at the Royal Institute of Technology (KTH). Moreover data from AL, OL and STD $CaCl_2$ methods were provided by the supervisors of this project for the overall data analysis.

Table 3: Extracting solution, SSR and time used for each soil P test.

Method	Extracting solution	SSR (g:mL)	Shaking time	Reference
1. Pi-filter strip	0.01 M $CaCl_2$ + Pi-paper strip	3.5:35	481 h	van Rotterdam *et al.*, (2009)
2. $CaCl_2$-depletion	0.01M $CaCl_2$	1:100	481 h	modified Houba *et al.*, (2000)
3. STD $CaCl_2$	0.01M $CaCl_2$	3:30	2 h	Houba *et al.*, (2000)
4. AL test	-	-	-	Egnér *et al*., (1960)
5. OL-test	-	-	-	Olsen *et al*., (1954)

3.3.1 P extraction using iron (hydr) oxide impregnated filter paper strips (Pi-strips)

Preparation of Pi-strips

Circular filter papers (150 mm diameter, Whatman 50) were moved fluently through an iron (III) oxide hexahydrate solution (0.4 M $FeCl_3 \cdot 6H_2O$) for 30 s, the process called impregnation. Thereafter, they were air-dried for 1h at room temperature. Dried papers were afterwards moved fluently though 5% ammonia solution for 30 s without interruption for neutralization of $FeCl_3$. The purpose of this process was to produce iron (II) oxide (FeO). The papers were immediately rinsed with demineralized water to remove adhering particles. Finally, they were cut into rectangular strips of 2 by 10 cm in size. In total 176 paper strips were made before the experiment.

Extraction of P from the soil-suspension

3.5 g dry soil was mixed with 35 mL 0.01 M $CaCl_2$ solution (1:10 soil solution ratio) in a 50 mL centrifuge tube. Four soils from each site were analyzed. Two samples were from control soils (unfertilized) and the other two were from highly fertilized soils. A strip was folded around a surface of a rectangular hollow holder positioned in a protective plastic container. The latter was perforated in its bottom to let the soil suspension reach the strip. Finally, the container holding a strip was placed inside the greiner tube (Figure 3) and covered. The designed system was then shaken continuously end-over-end at 6-rpm for 1 h. Thereafter, the Pi-strips were removed and replaced with new ones. The same shaking procedures were repeated after replacing the strips after 2, 4, 8, 24, 48, 78, 145, 221, 316, and 481 hours. The removed strips were thoroughly rinsed with distilled water to remove adhering particles immediately after removal and then air-dried. To desorb Pi-P, the strips were shaken with a 0.2 M H_2SO_4 20 mL solution for 2 h. Afterwards, the solutions in acid extract were filtered (Acrodisc PF Syringe Filter with 0.8/0.2 µm Super Membrane). The experiment was carried out in duplicate; therefore 16 soil samples were shaken at the same time. The concentration of P in the sulphuric acid solutions was analyzed in the laboratory of Aquatic Sciences and Assessment at SLU and the results were expressed in µg P L^{-1}. However, P values were reported in mg P kg^{-1} after unit conversion (Figure 4).

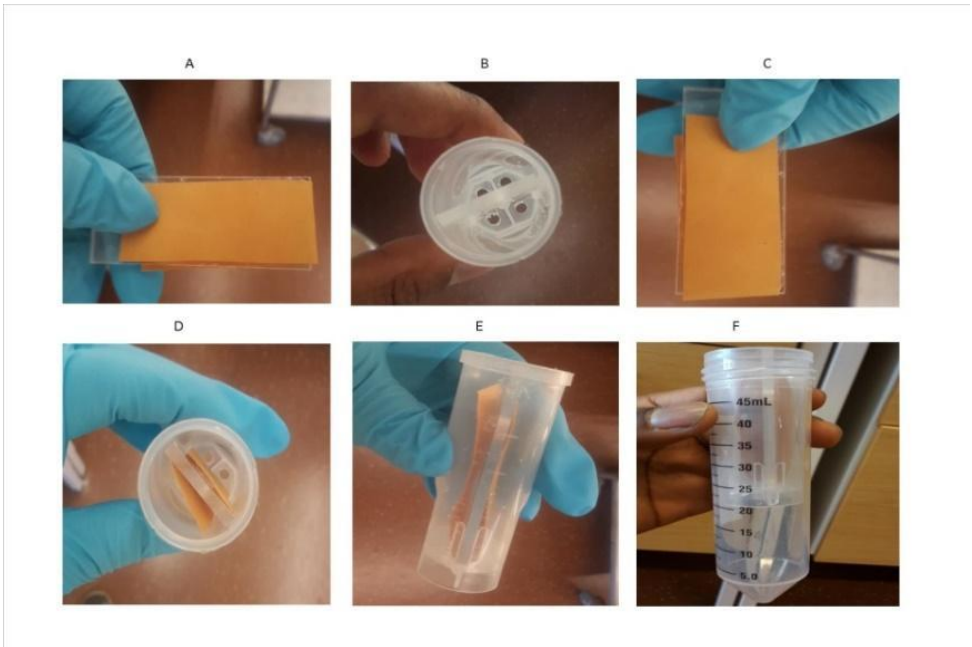


Figure 3: A laboratory experimental design system for shaking the Pi-strips with the soil suspension.

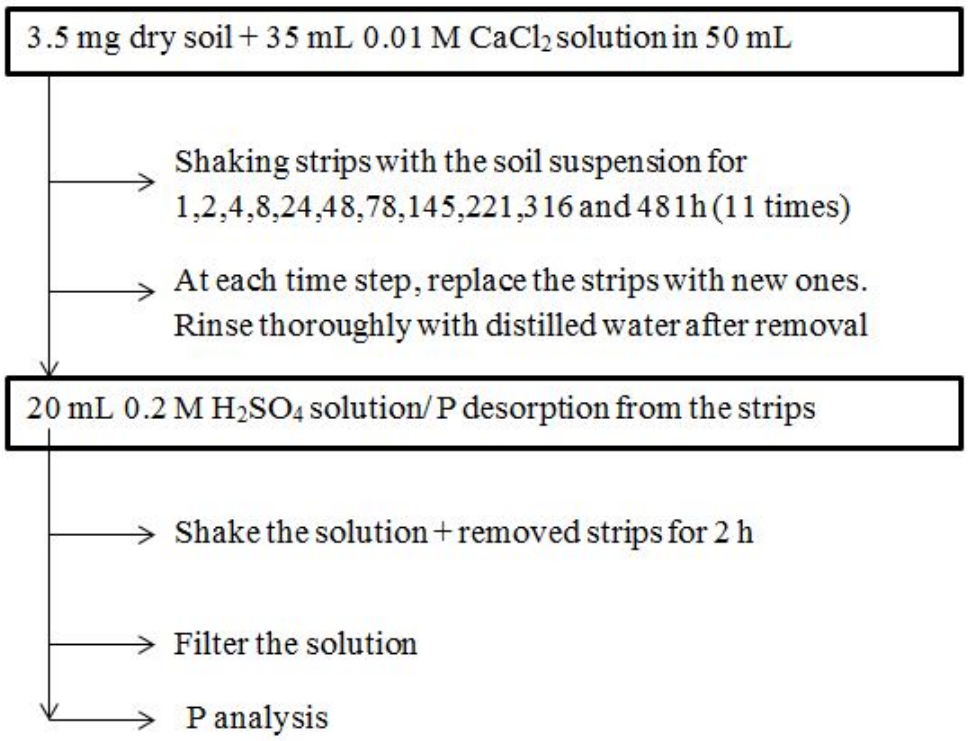


Figure 4: Laboratory procedures for shaking strips with the soil suspension and P desorption from the strips.

3.3.2 P extraction with 0.01 M $CaCl_2$ (depletion)

A 1-g sample of soil (dry weight basis) and 100 mL of 0.01 M $CaCl_2$ (0.01 soil: solution ratio) were mixed in a 250 mL polyethylene centrifuge bottle. For each site, 4 different soils in duplicates were analyzed, making 16 samples in total. The bottles were placed in an end-over-end shaking machine at 3 rpm. The samples were shaken for 1, 2, 4, 8, 24, 48, 78, 145, 221, 316, and 481 hours, successively. Additionally, each sample was manually shaken slightly before being placed in the shaker. After each time span, the soil samples had to be centrifuged for 15 minutes at 3000 rpm. 20 mL of the supernatant was collected and put into small plastic bottles for P analysis that was carried out at KTH using a Seal Analytical AA3 Autoanalyzer.

3.4 Statistical analysis

The experimental data from both methods were used to assess the Pi-filter strip extraction method and compare the results with the AL and OL-methods. Multivariate statistics was used for the statistical data analysis. Microsoft Excel 2010 was used to assess the amount of P extracted as a function of shaking time and the number of strips. Regression analysis was performed to assess the relationship and significance between tests. Interruption of shaking, the shaking intensity, interaction of strips with plastic containers and an uneven distribution of Fe (III) (hydr)oxide on filter papers were all factors that could affect the standard deviations.

Table 4: Background information on AL, OL and standard $CaCl_2$ methods (STD $CaCl_2$). P is expressed in mg kg-1. Data were provided by the supervisors. Standard $CaCl_2$ method measures the concentration of P as described by Houba et al., (2000). In this method, the soil to solution ratio = 0.1 and shaking time = 2h).

Sample ID	P test		
	AL	OL	STD $CaCl_2$
Ekebo			
EK A3-36	22.5	9	0.1
EK A3-55	27.5	7.5	0.0
EK D3-47	210	72	5.4
EK D3-62	215	75	7.8
Fjärdingslöv			
FJ A3-36	20	5.4	0.0
FJ A3-55	20	5	0.0
FJ D3-47	195	65	37.2
FJ D3-62	180	64	21.6

3 Results

The results of P extracted using the Pi-filter strip and $CaCl_2$-depletion method are shown in Table 5 and Table 6, respectively. These data represent the cumulative values of P extracted at each time step. The results show that the Pi-filter strip extraction method resulted in higher amounts of P than those found in the depletion experiment. In addition, the soils 1, 2, 5 and 6 have relatively very low P in both experiments.

Table 5: Cumulative amount of P adsorbed to 11 consecutive Pi-filter strips (mg P kg-1 soil).

		Extraction time (h)										
No	Sample ID	1	2	4	8	24	48	78	145	221	316	481
	Ekebo											
1	EK A3-36	0.8	1.8	2,8	4.6	7.2	9.5	11.7	13.7	15.6	17.3	19.0
2	EK A3-55	0.8	1,6	2.8	4.4	6.8	9.2	11.6	14.0	15.9	17.4	19.0
3	EK D3-47	6.3	11,9	19.3	28.7	44.3	61.7	78.8	100.6	120.1	138.7	164.7
4	EK D3-62	7.3	13,9	22.3	31.8	48.9	67.0	85.0	108.8	131.0	151.2	179.5
	Fjärdingslöv											
5	FJ A3-36	0.8	1,6	2.6	3.9	5.8	7.2	8.4	9.6	11.5	12.7	13.9
6	FJ A3-55	1.0	1,8	2.8	4.0	5.7	7.2	8.3	9.4	10.4	11.3	12.4
7	FJ D3-47	11.8	21,8	31.8	42.5	60.5	76.4	91.5	110.6	126.5	141.2	159.8
8	FJ D3-62	17.0	24,6	33.0	42.9	58.0	72.3	85.6	101.8	116.9	132.2	151.2

Table 6: Cumulative amount of P during the $CaCl_2$ -depletion extraction (mg P kg-1 soil).

		Extraction time (h)										
No	Sample ID	1	2	4	8	24	48	78	145	221	316	481
	Ekebo											
1	EK A3-36	0.0	0.0	0.0	0.5	0.7	1.9	1.9	1.9	1.9	1.9	2.1
2	EK A3-55	0.0	0.0	0.0	0.0	0.0	0.025	0.025	0.025	0.025	0.025	0.025
3	EK D3-47	1.3	4.6	7.1	8.8	10.7	12.9	14.4	15.9	17.5	18.8	20.4
4	EK D3-62	5.3	8.0	10.5	12.7	15.0	17.1	19.0	21.2	23.4	25.6	28.2
	Fjärdingslöv											
5	FJ A3-36	0.0	1.6	1.6	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
6	FJ A3-55	0.0	0.3	0.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
7	FJ D3-47	9.0	17.7	21.4	24.6	27.6	30.9	33.8	37.2	40.2	42.9	46.6
8	FJ D3-62	5.2	9.0	11.8	14.2	16.2	18.4	20.2	23.0	25.5	27.8	30.7

4.1 P adsorption on Pi- filter strips

4.1.1 Cumulative Pi-P

Cumulative P values were obtained by summing P adsorbed on individual 11 Pi-filter strips consecutively. Between all 8 soil samples, P removed by one strip during the first 1 h ranged between 4 and 11 % of the total Pi-P removed per soil sample. Between 27 and 46 % Pi-P was removed by the first 5 strips In total, 11 strips removed between 12 and 19 mg P kg^{-1} from P-deficient soils in 481 h and between 151 and 179 mg P kg^{-1} was removed from fertilized soil samples.

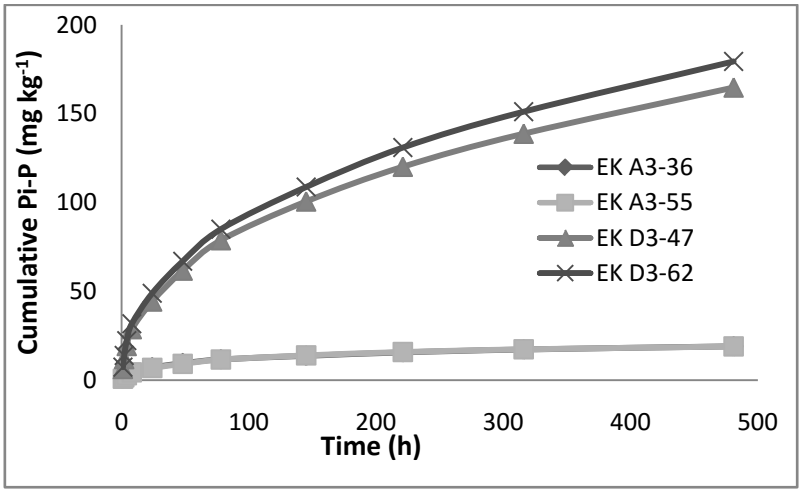


Figure 5: Cumulative amount of P adsorbed on 11 Pi-filter strips from Ekebo soil samples versus the amount of time.

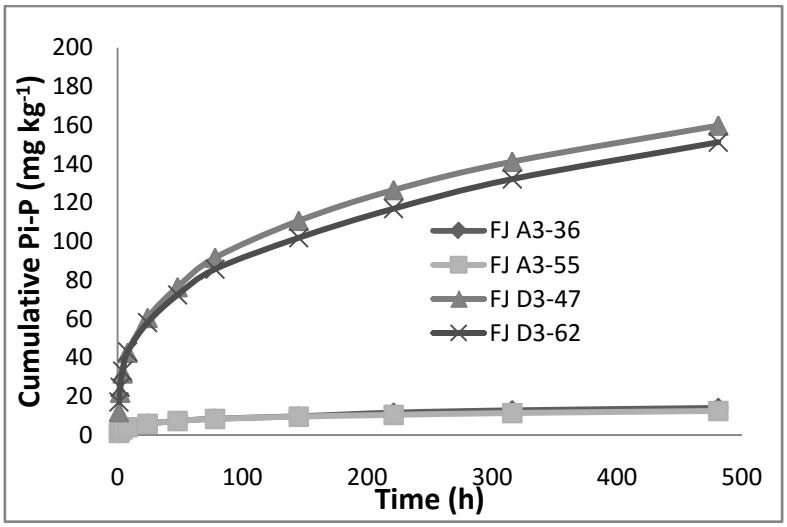


Figure 6: Cumulative amount of P adsorbed on 11 Pi-filter strips from Fjärdingslöv soil samples versus the amount of time.

When cumulative P is plotted against the number of filter strips, Pi-P was relatively low in the first 8 h. The Pi-P seemed to increase linearly as the number of filter strips increased per soil sample even though the curves turned into a different direction after 8 h (Fig. 7). In addition, the cumulative Pi-P was strongly correlated with the number of filter strips. The correlation coefficient (r) was between 0.97 and 0.99 (calculated for each soil sample data. The number of observations was 11).

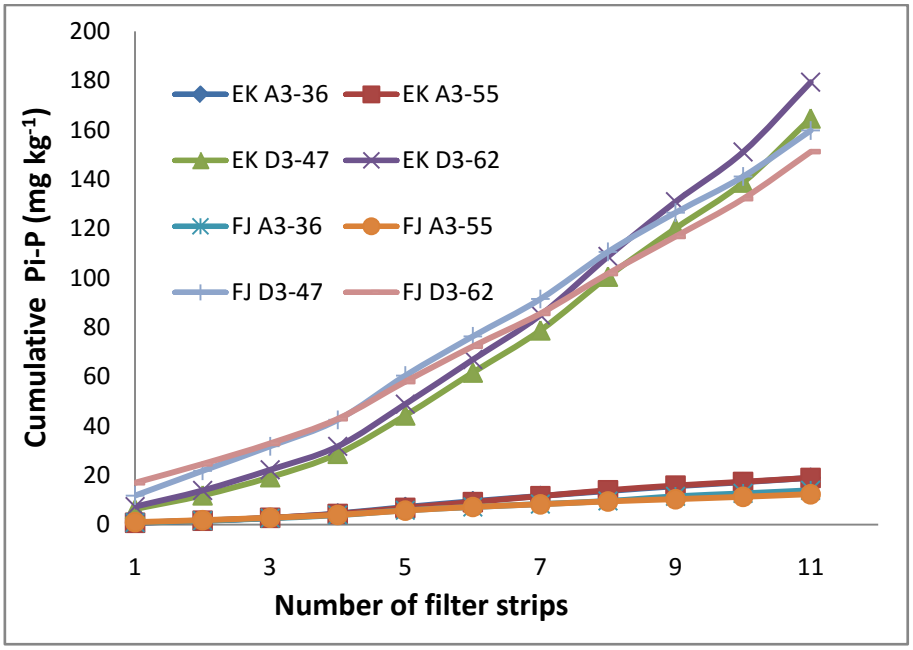


Figure 7: Cumulative amount of P adsorbed on 11 successive filter strips from 8 soil samples versus the number of Pi-filter strips.

4.1.3 P adsorbed on each individual Pi-strip

There was an increasing trend for the P adsorbed on the first 5 filter strips individually one after another in unfertilized soils (Fig. 8). Afterwards, it seemed to decrease generally over time. In highly fertilized soil samples, the trend was going upwards even though there was a switch between decrease and increase by considering the extraction time intervals separately. For example, in the last interval (between the 10^{th} and 11^{th} strips), the total P adsorbed to the last filter strips increased by 24 % from Pi-P of the 10^{th} strips in fertilized soils whereas from the 9^{th} to the 10^{th} strips, Pi-P dropped by 7%.

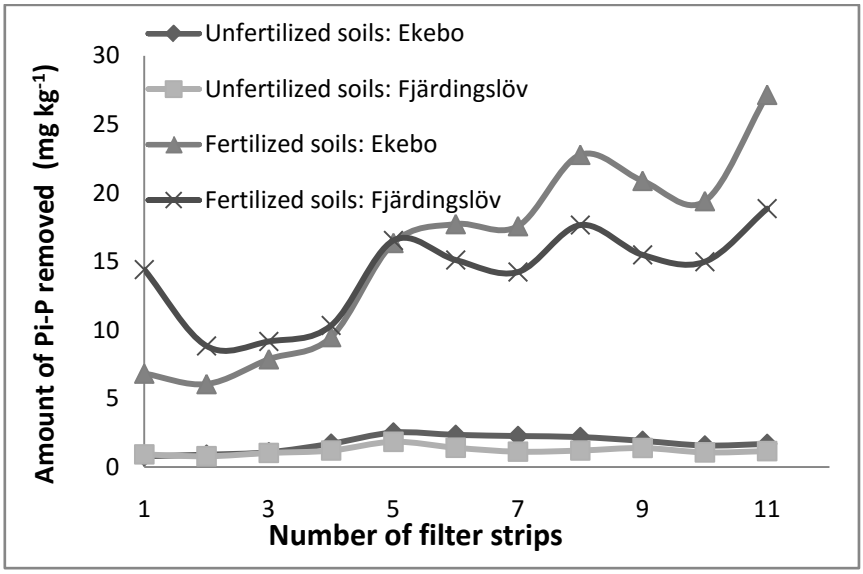


Figure 8: Amount of P adsorbed to individual Pi filter strips versus the number of filter strips.

4.2 Depletion of P by 0.01 M $CaCl_2$

As illustrated in Table 2, the soils chosen for this project differ in terms of their properties and soil P status. At both sites, there was a sharp increase in cumulative $CaCl_2$-P in the first 8 h before it started to slow down during the remaining time of extraction. Between the 1st and 2nd extraction events (1 and 2h), the cumulative amount of P removed increased by 50% whereas the extractable P increased by 8 % only between 316 and 481 h (10 and 11th extraction).

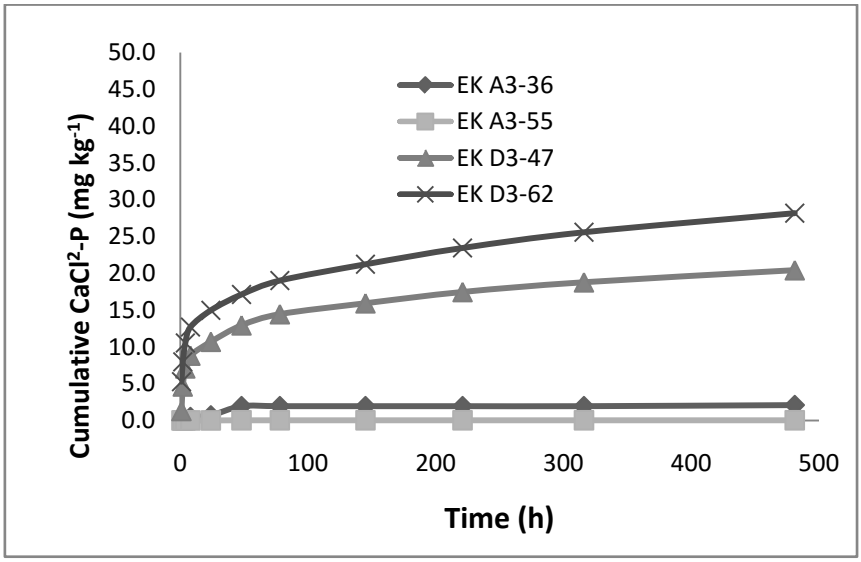
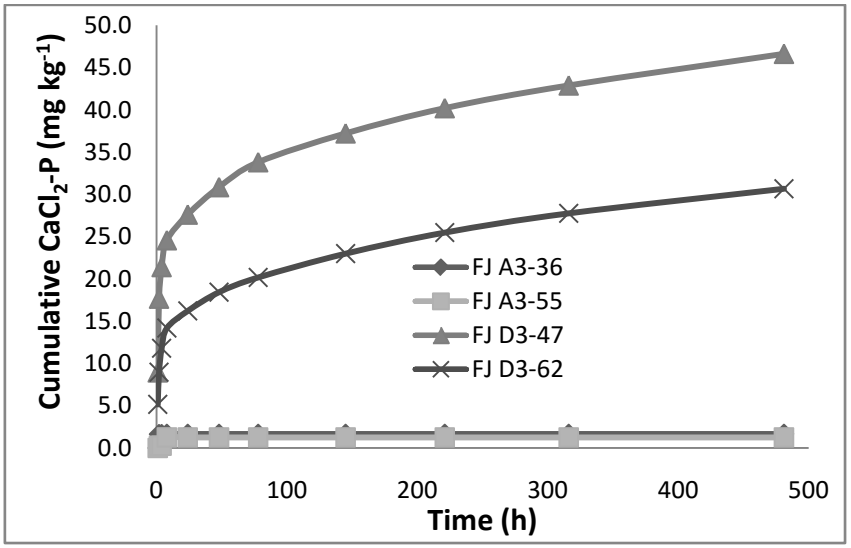


Figure 9: Cumulative amount of P extracted in 11 successive events versus the amount of time during the depletion experiment. Ekebo soils.



Figur 10: Cumulative amount of P extracted in 11 successive shaking events versus the time the samples were shaken during the depletion extraction. Fjärdingslöv soils.

4.4 Comparison between soil P tests

Regression analysis revealed that all soil P tests showed a strong positive correlation with one another except the data from a standard 0.01 M $CaCl_2$ (STD $CaCl_2$) extraction method (using the soil to solution ratio of 0.1 and shaking time of 2h). The data used in this statistical analysis were the total P extracted per soil for all 8 soil samples with their replicates (16 soil samples). P extracted by STD $CaCl_2$ was only strongly correlated with that measured during the $CaCl_2$-depletion experiment. From Table **7**, the starred R values (Linear correlation coefficients) are those for which the p-value < 0.05, showing a significant relationship between each another. The highest positive correlation was found between ($CaCl_2$-P; STD-$CaCl_2$-P), (Pi; AL), (Pi; OL) and (AL; OL) pairs.

*Table 7: Correlation coefficients (r) between Pi, AL, OL, $CaCl_2$-P and Initial P. * indicates a significant relationship between a combination of two tests (P < 0.05). Data used are total P extracted per each soil, number of observation: 16). Initial P is the concentration of P in the soil solution determined using the standard 0.01 M $CaCl_2$- method (Houba et al., 2000). A soil to solution ratio= 0.1, time= 2h.*

r-value	Pi-filter strip	AL	OL	$CaCl_2$-depletion
Pi-filter strip				
AL	0.998*			
OL	0.998*	0.998*		
$CaCl_2$-depletion	0.896*	0.887*	0.878*	
STD $CaCl_2$	0.665	0.654	0.638	0.919*

The highest amount of P was extracted by the AL followed by Pi-filter strip and OL (Fig. 11 and Fig.12). The P extraction efficiency decreased in order AL > Pi > OL > CaCl2-depletion) > STD $CaCl_2$ (Table 8). The average P extracted by the AL was 200 mg kg^{-1} in fertilized soils (D3) and 22.5 mg kg^{-1} in unfertilized soils (A3). The OL extracted the least amount of potentially available P of 68.7 mg kg^{-1} and 6.7 mg kg^{-1} in fertilized and unfertilized soils respectively.

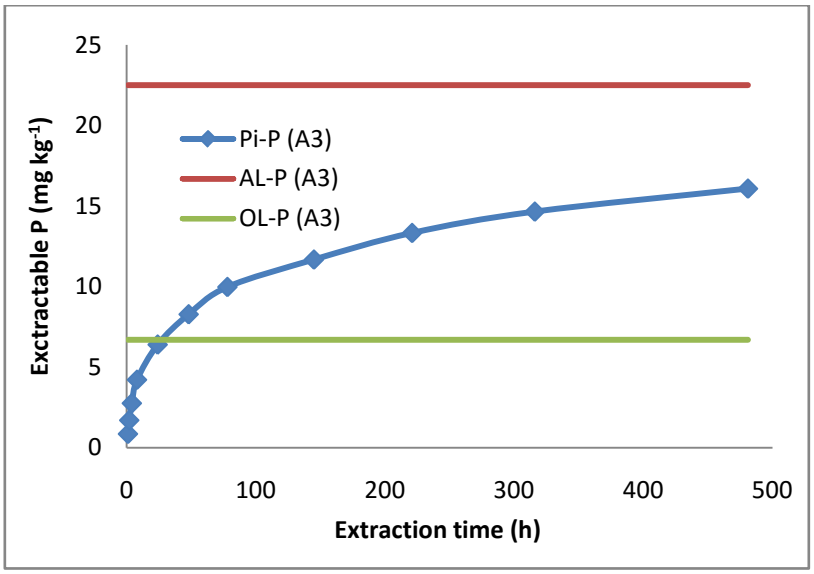


Figure 11: Average amount of P extracted from unfertilized soil samples (A3) using the Pi-, OL-, and AL-methods.

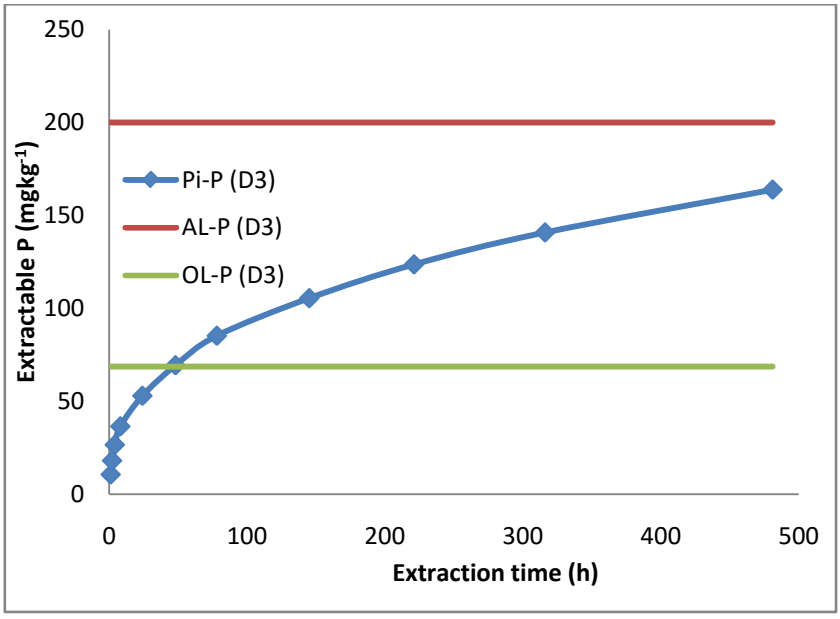


Figure 12: Average amount of P extracted from fertilized soil samples (D3) using the Pi-, OL-, and AL-methods

The P extracted by STD $CaCl_2$ and the P extracted during depletion experiments were very low in comparison to other methods. In fertilized soils, the initial P ranged between 2.6% and 19.1% of AL-P whereas in unfertilized soils this value was approximately 0. The P extraction efficiency decreased in the order Pi- > OL > $CaCl_2$- (depletion) > STD $CaCl_2$ methods

Table 8: P extraction methods Efficiency in relation to AL method.

		STD $CaCl_2$	$CaCl_2$-P	OL-P	Pi-P	AL-P
No	Sample ID	%				mg kg-1
	Ekebo					
1	EK A3-36	0.6	9.3	40	84.6	22.5
2	EK A3-55	0	0.1	27.3	69	27.5
3	EK D3-47	2.6	9.7	34.3	78.4	210
4	EK D3-62	3.6	13.1	34,9	83.5	215
	Fjärdingslöv					
5	FJ A3-36	0	8.3	27	69.7	20
6	FJ A3-55	0	6.3	25	61.9	20
7	FJ D3-47	19.1	23.9	33.1	82	195
8	FJ D3-62	12	17	35.3	84	180

5. Discussion

5.1 Evaluation of the P depletion and Pi-filter strip methods

The results from both experiments were able to explain the development of P desorption from the soil. In addition, they demonstrated the existence of dissimilarity between the two studied sites as shown in the previous studies (Eriksson *et al.*, 2015; Kirchmann *et al.*, 1999). However, some difficulties were encountered when attempting to implement these methods particularly during the Pi-filter strip method.

For the Pi-method to be successful, the filter papers should be evenly impregnated with iron (hydr)oxide solution (Chardon *et al.*, 1996). A filter paper should change its color from white to orange after preparation. In addition, it should be smooth at both sides. However, few of them seemed to have dark-orange spots in one of their sides. This could have been a result of the inconsistency during the movement of these papers through the $FeCl_3 \cdot 6H_2O$ solution or during the neutralization process with ammonia solution. If this was the case, it may result in a failure when preparing the Pi-filter papers. In other words, an uneven distribution of iron (III) oxide solution on the surface of the papers occurred. Moreover, during the drying process, the filter papers were placed on the paper towels. This was a feasible option found to minimize contamination during the drying process. Therefore, another possible explanation might be related to the fact that the particles from the tissue papers stuck on some of the filters during drying. It was also necessary to avoid completely the adhesion of the strips on the container walls. It needed regular checking as this happened often. Another consideration for this experiment resulted is the interchange of 2 strips that might have unintentionally occurred while drying. This happened among the strips removed from Ekebo soil after shaking for 8 hours.

For the depletion experiment, the shaking process was interrupted due to the shortage of the shaking machines. Therefore, some of the samples were kept in the refrigerator for a couple of days. It is recommended that the storage of soil samples for the determination of P in solution could be best at the temperature below 4^oC (Houba *et al.;* 2000). Even though those samples were stored at that temperature or even slightly below, this could have had an influence since some of them were stored for about 5 to 6 days. Moreover, some soil samples were shaken at different speeds due to the use of different machines during the depletion experiment. This inconsistency for the shaking process might have had a slight effect on the results.

5.2 P extracted by the Pi-filter strip and depletion methods

5.2.1 Cumulative P

In the Pi-method, the P-removal curves were steeper than in depletion experiment. This means that the strips had much more potential to extract the adsorbed P than the 0.01 M $CaCl_2$-solution. The P desorption rate was higher in the beginning and slowed down with time. This reflects the P desorption behavior (Lookman *et al*., 1995; Rotterdam *et al*., 2009; Rotterdam *et al*., 2012). As P is fast removed from the soil solution, it is rapidly replenished by the most easily soil-adsorbed P, which is in turn refilled by the strongly bound P in soil (Sparks *et al*., 1996). In the presence of P sinks such as Pi-filter strips, the P desorption from the soil is enhanced thus increasing the chance for slow desorption rate to take place. In a short time, the rate of P adsorption to the strips could be believed to control the transfer of P from the soil to the strips. The reason for that is that the equilibrium between the soil-adsorbed P and the solution P is constantly attained at each time P is removed from the solution (Rotterdam *et al*., 2009). After maximum desorption have reached, this changed thus slowing down the rate of desorption. Studies by Rotterdam *et al*. (2009) showed that the maximum rate of P desorption was reached after 24 h. In that study, the determination of the solution P and Pi-methods were carried out together. This helped to identify the point whereby the maximum desorption was reached. It seems impossible to accurately confirm this point in this study. We may instead rely solely on the curves shown in Fig. 9 and Fig. 10 according to which the highest P removal occurs after 8 h during the depletion experiment. In the Pi-filter strip method; this point seems to take place later on even though this was hard to see it on Fig. 5 and Fig. 6.

A strong relationship was found between the number of strips and the cumulative amount of P adsorbed to the Pi strips (Fig. 7). This suggests that P desorption is also enhanced by the frequency of extractions. As the number of strips increased, more P was removed from the soil. Studies by Rotterdam *et al*. (2009) showed also that the cumulative Pi-P increases linearly with the number of strips.

Furthermore, the data from the standard 0.01 M $CaCl_2$-method (STD $CaCl_2$) helped to explain the effect of changing the soil to solution ratio (SSR) and the length of extraction time during the depletion experiment. A standardized method to characterize the P in solution of the soil is based on shaking the soil solution samples with the ratio of 0.1 during 2 h (Houba *et al*., 2000). The extractable P with this method was much less than the total P extracted during the $CaCl_2$-depletion experiment. This reveals that more P can be extracted by increasing the extraction time. However, the STD $CaCl_2$-method resulted in higher P than that

extracted during 2 h of the depletion experiment. A possible explanation for this might be that increasing the volume of the extractant solution resulted in low solution P, as a lower soil to solution ratio (1: 100) was used in the depletion experiment.

5.2.2 The amount of P extracted on individual strips

P adsorbed on individual strips during the sequential extraction depends on the soil P fertility level and the length of desorption time as shown in Fig. 8. In P-deficient soils, the highest amount was removed by the fifth Pi-filter strips. Afterwards, the amount of P adsorbed by individual strips seemed to decrease slightly one after another. The reason for this could be that only few filter strips are sufficient to deplete the easily soil-adsorbed P in these soils. On the other hand, the extractable P increased with the succession of strips in fertilized soils, but fluctuations occurred between some points of extraction. This shows that the length of contact time between the strips was very important for the P releasable quantity.

5.3 Difference between the treatments as affected by long-term P fertilization.

Ekebo soil has the highest potentially available but less soil P in solution in comparison to Fjärdingslöv. This difference is explained by the diversity in their soil properties as shown in Table 2. The X-ray adsorption near-edge structure (XANES) spectroscopy indicated that the soil of Ekebo consists of higher concentration of oxalate-extractable Al and Fe as well higher organic C in the clay fraction. In addition, the proportion of kaolinite in the clay mineralogy is higher than in Fjärdingslöv in the topsoil (Eriksson *et al.*, 2015). Therefore, more P is adsorbed on Al and Fe (hydr)oxides in comparison with the soil of Fjärdingslöv. This fraction of P can be released into the solution by the strong extractants such as Pi-filter strips. In addition, the higher organic matter content can explain also that Pi-method extracted more P from the Ekebo soil (Eriksson *et al.*, 2015).

The data from the $CaCl_2$-depletion experiment revealed that the solution P in the unfertilized soils was generally below the detection limit. In addition, the fertilized soils fall in the category of P-deficient soils. This seems to be incorrect since some of these soils have been extensively fertilized. In P-deficient soils, the concentration varies from 0.31 to 31 µg L^{-1}, and can reach 3100 µg L^{-1} in P-rich soils (Syers et al., 2008). These results are likely to be related to the strength of 0.01 M $CaCl_2$ solution as an extractant. In the master thesis of Jarosch (2012), it was found that water can release between 24 to 68 % of AL-P from various soils after consecutive extractions. In this study, only 24% of AL-P was extracted by

0.01 M $CaCl_2$ in one of 8 soils studied. For the other soils, the percentage ranged between 0.1 and 13% after 11 successive extractions. Moreover, the solution P of soil sample 2 (EK A3-55) was much less than that of soil 1 (EK A3-36), yet they are all unfertilized from the same site. It is difficult to explain this result, but it can be related to insufficient data. Most of the data about the soil properties (oxalate-extractable Al and Fe) were average values for the entire site. It may be possible that some plots may be different from others within the same site. It would have been better to have more data about the specific soil properties for each single plot.

5.4 P-extraction efficiency between soil P tests

All soil P tests showed that Ekebo has a higher extractable P than Fjärdingslöv except 0.01 M $CaCl_2$-methods. This emphasizes the importance of the soil P sorption capacity (Eriksson *et al.*, 2015; Kirchmann *et al.*, 1999).

The results of this study indicate also that the AL was able to extract more P from the soil followed by the Pi-method. The efficiency of P extraction decreased in the order AL- > Pi > OL > $CaCl_2$-depletion > STD $CaCl_2$. The r values (Table 7) found between pairs of the first three tests indicates that they possibly have the same potential to extract the soil-adsorbed P. Similarly, all the first three soil P tests showed similar potential to indicate the removal from the soil to the solution, especially the Pi filter strip method. This is shown by the r values between each of them and the depletion method. However, these findings may not be much encouraging given that the correlation analysis depended on only duplicates of 8 soil samples (16 samples). The correlation might be much more reliable if many samples were used.

Many of the studies comparing the efficiency between soil P tests showed that OL-method results in higher extractable P than Pi-P (Neyroud & Lischer, 2003; Indiati & Singh, 2007; Wuenscher *et al.*, 2015). The soil suspension was shaken for less than 24 h in the Pi-filter strip method, whereas this method was carried out in 481 h resulting in higher P. Therefore, the higher Pi-P than OL-P could have been influenced by the dissimilarity in shaking times.

There was clearly also a relationship between the AL-P and OL-P but OL-P was only between 25 and 40 % of the AL-P for all soils studied (Table 8). In the AL extraction, the P desorption process takes place through the dissolution of Al and Fe (hydr)oxides at low pH (Jordan-Meille *et al.*, 2012, Eriksson, 2016). It can also dissolve Ca-phosphates, thus overestimating the pool of surface-active adsorbed P

(Otabbong *et al.*, 2009). On the other hand, HCO_3^{2-} and CO_3^{2-} of the OL method may precipitate with metals ions thus releasing phosphates in the solution especially in calcareous soils (Jordan-Meille *et al.*, 2012, Eriksson, 2016). However, the pH in all our soil samples was around neutral. At this pH, the solubility of P is mostly controlled by adsorption-desorption processes (Hinsinger, 2001). There was no clear effect of pH in these soils to explain the difference between these two methods. Finally, the P adsorbed on the strips was between 62% and 86% of the AL-P in all soils studied (Table 8). It was previously noticed that 481 h shaking time was not enough to deplete the potentially available P from the soil. Therefore, the length of shaking time could be the main reason for the low extractable Pi-P than AL-P.

The ratios between OL-P and AL-P, and Pi-P and AL-P were more or less the same in fertilized soils between the two sites (Table 8). On the other hand, these ratios were slightly higher in unfertilized soils of Ekebo compared with their corresponding Fjärdingslöv unfertilized soils. It is therefore likely that the effect of $CaCO_3$ on AL-P can be seen in unfertilized soils as a sign of P overestimation.

In summary, there was no effect of soil properties such as pH and the soil texture, found to explain the difference between AL-P, OL-P and Pi-P. However, the composition of each extractant, and the length of shaking time could probably be the reasons to explain the results.

5.5 The limitations

This project was conducted mainly to study P extractability from Ekebo and Fjärdingslöv sites using the Pi-filter strip method. An additional experiment of using the P depletion method was also carried out. Finally these methods were evaluated using AL, OL and the standard 0.01 M $CaCl_2$-method.The laboratory work was conducted at SLU and KTH. This study was limited by the number of duplicates used to fully study the P availability. In addition, only the soil samples collected were those representing the lowest and highest P status in the Swedish long-term fertility experiments. The reason for these limitations was mainly because of the normal time span for writing the thesis. Another point to consider is the discontinuity in shaking process during the depletion experiment. The machine had a limited capacity to handle all samples at the same time. Some samples were shaken end-over-end while others were shaken horizontally during the depletion experiment.

5.6 Comparison with the studies of Rotterdam et al. (2009) and Rotterdam et al., (2012).

The objective of this study was to evaluate P extraction using the Pi-filter strips in the soils included in the Swedish long-term fertility experiments. Our experiments were performed based on the procedures used in the studies of (Rotterdam *et al*., 2009; Rotterdam *et al*., 2012). The soil samples 2 and 5 from those studies, with much higher Pi-P are possibly, highly fertilized since they have even a very higher P concentration in solution (Table 9). We can therefore compare these soils with the fertilized soils in this report. On the other hand, those with the lowest Pi-P have resulted in very low P concentration in solution and high oxalate-extractable Al and Fe in most of the studied soils from Netherlands. These may be comparable with our P-deficient soils. It is hard to fully compare the difference with those studies since the soils analysed are different. All soil samples were shaken similarly for an equal length of time except that during our experiment, the 10^{th} strip was replaced from the soil suspension after 316 h as compared to 337 h in those studies. Therefore, this could partly explain why we got relatively lower P values during the first 10 consecutive extractions. A more clear comparison can otherwise be made if these soils were the similar.

Although our study used soils with different sites and properties, the investigated method seemed to work well as the results from different studies showed the P values which are close in magnitude (range).

Table 9: Comparison between (Rotterdam et al., 2009; Rotterdam et al., 2012) and this study. Cumulative P adsorbed to 10 consecutive Pi-filter strips.

	This study	(Rotterdam *et al.*, 2009; Rotterdam *et al.*, 2012)				
No	(mmol/kg^{-})Pi-P	mmol kg^{-1}Pi-P	mmol kg^{-1} $(Al+Fe)_{0x}$	pH	% $CaCO_3$	µmol kg^{-1} $CaCl_2$-P
1	0.6	3.6	81	5	-	246
2	0.6	8.9	71	6	-	646
3	4.6	3.9	92	5.5	-	58
4	5	2.4	361	5.6	-	35
5	0.4	8.6	70	6.7	-	809
6	0.4	1.6	12	6.7	0.6	161
7	4.7	4.1	65	5.1	-	162
8	4.4	3.2	69	5.5	-	163
9	-	2.9	239	4.2	-	77
10	-	1.1	34	6.9	1.7	39
11	-	2.2	307	5.3	-	22
12	-	2.4	50	7.5	1.7	115
13	-	1.6	233	4.7	-	19
14	-	1.6	106	7.1	6.5	26
15	-	2.2	97	7.3	2.8	45
16	-	1.7	13	7.2	0.7	209
17	-	2	282	6	-	19

5.7 Future consideration

To maximize its efficiency, improvement on the position of the strips while shaking is suggested to avoid adhesion to the walls of the tubes. It is also suggested to study the rate of P desorption soil as a result of removal by the strips from the soil solution. This may be achieved by measuring the P concentration in solution at each time the strip is removed and after the equilibrium is established between the solution and the soil-adsorbed P. Moreover, to better investigate the efficiency of the Pi-filter strip method in comparison with the AL-method, further study may include a wide range terms of soil properties ranging from acidic to alkaline soils .

6. Conclusion

- The laboratory experiments designed to carry out both the P depletion and sequential extraction of soil-adsorbed P were reasonably successful. These methods were able to explain the P desorption behavior.

- Additional extraction time is needed for the Pi-filter strip method to determine the potentially available pool of P. The length of desorption time and the number of strips per soil were very important to characterize the soil-adsorbed P for the soils studied.

- There were large differences between the investigated methods in terms of the potentially available P. AL extracted much more P than OL. The results of the Pi filter strip method were more in accordance with the AL method, suggesting the latter may be preferred over the OL method for estimating the potential availability of P in the short term.

- In soils with pH around neutral and non-calcareous soils, it is difficult to evaluate the efficiency of the Pi-filter strip method based on the P extractability using the AL and OL methods. A larger diversity of soils could be helpful to fully evaluate these different soil P tests.

- The concentration level of Al, Fe (hydr)oxides and the organic matter has a great impact on extractable P in the long-term fertilization.

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Appendices

Appendix 1: Average amount of P ($mgkg^{-1}$) removed during the $CaCl_2$ depletion experiment at each extraction step including the standard deviations.

		Extraction time										
No	Sample ID	1h	2h	4h	8h	24h	48h	78h	145h	221h	316h	481h
	Ekebo											
1	EK A3-36	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.05	0.2 ± 0.1	1.2 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2
2	EK A3-55	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.02 ± 0.03	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3	EK D3-47	1.3 ± 0.7	3.3 ± 1.2	2.5 ± 0.44	1.8 ± 0.2	1.9 ± 0.1	2.2 ± 0.7	1.5 ± 0.1	1.5 ± 0.2	1.5 ± 0.7	1.3 ± 0.04	1.6 ± 0.2
4	EK D3-62	5.3 ± 3.7	2.7± 0.04	2.6 ± 0.1	2.1 ± 0.3	2.3 ± 0.1	2.1 ± 0.02	1.9 ± 0.07	2.2 ± 0.1	2.2 ± 0.0	2.1 ± 0.0	2.6 ± 0.1
	Fjädringslöv											
5	FJ A3-36	0.0 ± 0.0	1.6 ± 2.3	0.0 ± 0.0	0.03 ± 0.05	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
6	FJ A3-55	0.0 ± 0.0	0.3 ± 0.4	0.0 ± 0.0	0.9 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
7	FJ D3-47	8.9 ± 0.0	8.7 ± 4.8	3.7 ± 0.1	3.2 ± 0.0	3.0 ± 0.0	3.2 ± 0.2	2.9 ± 0.4	3.4 ± 0.2	2.9 ± 0.1	2.7 ± 0.1	3.7 ± 0.3
8	FJ D3-62	5.2 ± 0.4	3.8 ± 0.07	2.8 ± 0.5	2.4 ± 0.1	2.0 ± 0.2	2.2 ± 0.0	1.8 ± 0. 1	2.8 ± 0.1	2.5 ± 0.0	2.3 ± 0.0	2.9 ± 0.1

Appendix 2: Average amount of P ($mgkg^{-1}$) removed by individual filter strips including the standard deviations.

		Extraction time										
No	Sample ID	1h	2h	4h	8h	24h	48h	78h	145h	221h	316h	481h
	Ekebo											
1	EK A3-36	0.8 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	1.8 ± 0.7	2.6 ± 0.4	2.4 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.3	1.6 ± 0.4	1.8 ± 0.2
2	EK A3-55	0.8 ± 0.0	0.9 ± 0.01	1.2 ± 0.0	1.6 ± 0.06	2.4 ± 0.1	2.3 ± 0.0	2.4 ± 0.2	2.4 ± 0.0	1.9 ± 0.3	1.5 ± 0.1	1.6 ± 0.1
3	EK D3-47	6.0 ± 0.3	5.5 ± 0.1	7.4 ± 0.5	9.5 ± 1.4	15.6 ± 1	17.4 ± 0.1	17.0 ± 0.8	21.8 ± 2	19.6 ± 1.5	18.6 ± 0.6	26.0 ± 0.8
4	EK D3-62	7.0 ± 0.2	6.6 ± 0.1	8.4 ± 0.1	9,5 ± 0.8	17.1 ± 0.3	18.0 ± 0.1	18.1 ± 1	23.8 ± 0.1	22.2 ± 0.4	20.2 ± 0.1	28.3 ± 1.1
	Fjärdingslöv											
5	FJ A3-36	0.8 ± 0	0.8 ± 0.0	1.0 ± 0	1.2 ± 0.1	2 ± 0.2	1.4 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	1.9 ± 1.3	1.2 ± 0.2	1.3 ± 0.1
6	FJ A3-55	1.0 ± 0.2	0.8 ± 0.0	1.0 ± 0.0	1.1 ± 0	1.8 ± 0.1	1.5 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	09 ± 0.0	1.0 ± 0.2	1.1 ± 0.0
7	FJ D3-47	11.8 ± 0.2	10.0 ± 0.4	10.0 ± 0.0	10.7 ± 0.1	18.0 ± 0.1	15.9 ± 0.3	15.1 ± 0.1	19.1 ± 0.9	15.9 ± 0.0	14.7 ± 0.2	18.7 ± 0.6
8	FJ D3-62	17 ± 2.9	7.7 ± 0.05	8.0 ± 0.2	10.0 ± 1.3	15.1 ± 1.4	14.3 ± 0.4	13.3 ± 0.1	16.2 ± 0.6	15.1 ± 1.3	15.3 ± 0.1	19.1 ± 0.9